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### **REMARKS**

#### **The Invention.**

The present invention provides *methods* for improved protein production from a cell culture using culture components and conditions that dramatically increase the amount of protein produced. The improved methods can be used for the production of proteins encoded by naturally occurring cellulase genes as well as from various heterologous constructs.

The claimed invention requires that the method use an inducing feed composition as a component of the feed stream (either batch or continuous) to induce cellulase production or proteins under the control of certain promoters. Lactose is the usual carbon source used in the production of cellulases. Sophorose is the most potent inducer of cellulase expression. Glucose, the main component of the inducing feed composition, although less expensive than both lactose and sophorose, represses cellulase expression. The inventors have found that the production costs of proteins regulated by certain promoters can be reduced by using an *in situ* cellulase-treated concentrated glucose solution described by the present invention.

#### **Status of the Application.**

Claims 15-32, 34 and 35 are pending in the application. Claims 15 has been amended herein. Claims 36 – 40 are new. Claim 15 has been amended to recite components in the inducing feed composition. Support for this amendment may be found throughout the specification as filed and particularly at page 3 and 6. Applicants assert new matter has not been introduced by the amendment.

#### **Claim Objections.**

Claim 15 has been objected to. Specifically, the Examiner asserts that the "a proteins" should be corrected to read "a protein". Applicants note that the "s" at the end of "proteins" had been deleted in the last amendment filed. However, Applicants believe that it may not have been clearly visible due to the facsimile transmission and have, therefore, made the correction explicit by using the double brackets around the "s".

Withdrawal of the objection is respectfully requested.

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**35 U.S.C. §102.**

It is well-settled law that to anticipate a claim the prior art reference must contain each and every element within the four corners of the document. Thus, Applicants submit that there can be no anticipation unless all of the same elements of the invention are found within the four corners of a single reference. *Lewmar Marine, Inc. v. Barient, Inc.*, 827 F.2d 744, 747, 3 USPQ2d 1766, 1767-68 (Fed. Cir. 1987). A reference that merely contains substantially the same elements or only broadly teaches the invention is insufficient to establish anticipation. *Jamesbury Corp. v. Litton Industrial Products, Inc.*, 756 F.2d 1556, 1560, 225 USPQ 253, 256 (Fed. Cir. 1985). The Federal Circuit has stated that "anticipation does not require actual performance of suggestions in a disclosure. Rather, anticipation only requires that those suggestions be enabling to one of skill in the art." *Bristol-Myers Squibb Co. v. Ben Venue Laboratories Inc.*, 246 F.3d 1368, 1379, 58 USPQ2d 1508, 1516 (Fed. Cir. 2001). Thus, "even if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling." *In re Donohue*, 766 F.2d 531, 533, 226 USPQ 619, 621 (Fed. Cir. 1985), *citing In re Borst*, 345 F.2d 851, 855, 45 USPQ 554, 557 (CCPA 1965), *cert. denied*, 382 U.S. 973, 148 USPQ 771 (1966).

At the time the present application was filed, it was standard practice in the art to use lactose in fermentations of microorganisms producing proteins of interest under the control of cellulase promoters to induce expression. See, for example, Seiboth *et al.*, *Mol. Genet. Genomics* (2002) 267:124-132, attached hereto. Therein it is stated "Lactose is at present the only soluble carbon source which can be used economically for the production by *Hypocrea jecorina* (= *Trichoderma reesei*) of cellulases or heterologous proteins under the control of cellulase expression signals."

Applicants also attach hereto a copy of Ilmen *et al.* (*Appl. Environ. Microbiol.* (1997) 63 (4):1298-1306) which clearly states that "no expression could be observed on glucose-containing medium and that high glucose levels abolish the inducing effect of sophorose" (see Abstract).

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**35 U.S.C. §102(b).**

Claims 15-28 and 31-32 stand rejected under 35 USC §102(b) as being anticipated by Mitchinson *et al.* (US Pat. No. 6,268,328). Specifically, the Examiner asserts that Mitchinson *et al.* teaches all the limitations of the claimed invention.

Applicants have amended claim 15 to recite the composition of the inducing feed. As noted above, the claimed invention uses an inducing feed composition as a component of the feed stream to induce protein production.

The Examiner has urged that "since the claim does not recite the specific components of the 'inducing feed composition' it is deemed...that it is equivalent to the culture media disclosed in the cited reference." Although Applicants (still) believe this is an untenable statement, Claim 15 has been amended to recite the components in the inducing feed. This is clearly not disclosed in Mitchinson *et al.*.

First, media for the cultivation of microorganisms contains the substances necessary to support the growth of microorganisms including a carbon source for incorporation into cell biomass. Second, fermentation conditions can be optimized to provide for the maximum increase in biomass utilizing glucose. However, as noted above, **glucose is a known repressor** of cellulase expression. If the Examiner is asserting that the media used in Mitchinson *et al.* contains between about 5% and 75% glucose and induces cellulase expression, then Applicants assert that Mitchinson *et al.* fails to provide any details on the growth medium. Although Mitchinson *et al.* states that "Any growth medium can be used in the present invention that is suitable to grow the desired transformants" and refers to a "liquid media" it thoroughly lacks any guidance on the components of such a medium. It should also be noted that growth medium contains all that is needed by the cells to grow and that there is no need for the microorganism to produce enzymes that are normally produced in response to a lack of a specific nutrient, e.g., glucose. Furthermore, in the fermenter for enzyme production, the culture or nutrient medium comprises mineral salts, usual vitamin complements and surfactants to which is added an inducer of cellulases in order to optimize the cellulase production. In the present method, the inducing feed is used as a replacement to the usual amount of cellulosic inductor (e.g. purified cellulose, paper pulp, lactose etc.). At the time Mitchinson was written it was standard procedure to use lactose as the inductor in

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fermentations. Applicants provide herewith numerous journal articles stating that at times later than 1998 that the cells were grown in medium that contained lactose as the inductor and not a mixed sugar inducing feed as presently claimed. The instant invention simply isn't within Mitchinson's disclosure.

Mitchinson *et al.* is not only silent on the addition of an inducing feed composition but also on the critical aspect of how they were able to induce protein production in the presence of glucose. Thus, Mitchinson *et al.* fails to provide an enabling disclosure and fails as prior art as noted above. See *Bristol-Myers Squibb Co. and In re Donohue*.

Finally, Example 2 on pages 23 and 24 of the instant specification shows that it is not the medium but the inducing feed composition that induces protein production when the cells are grown under otherwise identical conditions. Therefore, one skilled in the art would not have found the instant invention in the cited art and (all of) the cited references fail to anticipate the claimed invention.

Withdrawal of the rejection is respectfully requested.

**35 U.S.C. §102(e).**

*Claims 15-28, 31-32 and 34-35*

Claims 15-28, 31-32 and 34-35 stand rejected under 35 USC §102(e) as being anticipated by Fowler *et al.* (US Pat. No. 6,407,046). In addition, Claims 15-18 and 23-29 stand rejected under 35 USC §102(e) as being anticipated by Lehmbeck (US Pat. No. 6,352,841). Specifically, the Examiner asserts that Fowler *et al.* and Lehmbeck each teach all the limitations of the claimed invention.

For the reasons given above related to the Mitchinson *et al.* reference, Applicants assert that the Fowler *et al.* and the Lehman references each fail to anticipate the claimed invention. The disclosure of Fowler *et al.* is virtually identical to the Mitchinson *et al.* disclosure. Lehmbeck, at best, states that "A mixture of 20% maltose liquor and 8% urea was added continuously; phosphoric acid was added as necessary to maintain a pH of 7" but states nothing about using a concentrated glucose solution. Therefore, neither one provides any reason to believe that they disclose, let alone enable, an inducing feed composition.

Withdrawal of the rejections is respectfully requested.

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**CONCLUSION**

In light of the above amendments, as well as the remarks, the Applicants believe the pending claims are in condition for allowance and issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 846-7615.

Respectfully submitted,

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